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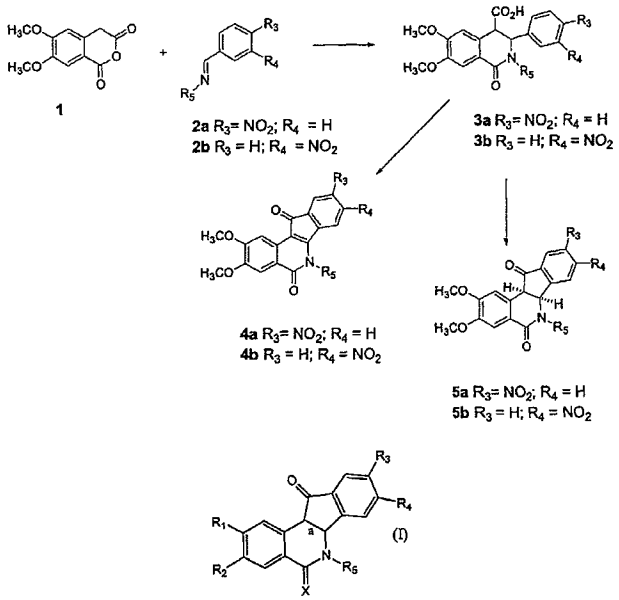
(54) **Title:** NITRO AND AMINO SUBSTITUTED HETEROCYCLES AS TOPOISOMERASE I TARGETING AGENTS



(57) **Abstract:** The invention provides compounds of formula (I): wherein: R_1-R_5, "a" and X have any of the meanings defined in the specification and their pharmaceutically acceptable salts. The invention also provides pharmaceutical compositions comprising a compound of formula (I), processes for preparing compounds of formula (I), intermediates useful for preparing compounds of formula (I), and therapeutic methods for treating cancer using compounds of formula (I).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

NITRO AND AMINO SUBSTITUTED HETEROCYCLES AS TOPOISOMERASE I TARGETING AGENTS

Priority of Invention

This application claims priority to United States Provisional Patent Application Number 60/402166, filed 09 August 2002.

Government Funding

The invention described herein was made with government support under Grant Numbers CA39662 and CA077433 from the National Cancer Institute. The United States Government has certain rights in the invention.

Background of the Invention

DNA-topoisomerases are enzymes which are present in the nuclei of cells where they catalyze the breaking and rejoining of DNA strands, which control the topological state of DNA. Recent studies also suggest that topoisomerases are also involved in regulating template supercoiling during RNA transcription. There are two major classes of mammalian topoisomerases. DNA-topoisomerase-I catalyzes changes in the topological state of duplex DNA by performing transient single-strand breakage-union cycles. In contrast, mammalian topoisomerase II alters the topology of DNA by causing a transient enzyme bridged double-strand break, followed by strand passing and resealing. Mammalian topoisomerase II has been further classified as Type II α and Type II β. The antitumor activity associated with agents that are topoisomerase poisons is associated with their ability to stabilize the enzyme-DNA cleavable complex. This drug-induced stabilization of the enzyme-DNA cleavable complex effectively converts the enzyme into a cellular poison.

Several antitumor agents in clinical use have potent activity as mammalian topoisomerase II poisons. These include adriamycin, actinomycin D, daunomycin, VP-16, and VM-26 (teniposide or epipodophyllotoxin). In

contrast to the number of clinical and experimental drugs which act as topoisomerase II poisons, there are currently only a limited number of agents which have been identified as topoisomerase I poisons. Camptothecin and its structurally-related analogs are among the most extensively studied topoisomerase I poisons. Recently, bi- and terbenzimidazoles (Chen et al., *Cancer Res.* **1993**, *53*, 1332-1335; Sun et al., *J. Med. Chem.* **1995**, *38*, 3638-3644; Kim et al., *J. Med. Chem.* **1996**, *39*, 992-998), certain benzo[c]phenanthridine and protoberberine alkaloids and their synthetic analogs (Makhey et al., *Med. Chem. Res.* **1995**, *5*, 1-12; Janin et al., *J. Med. Chem.* **1975**, *18*, 708-713; Makhey et al., *Bioorg. & Med. Chem.* **1996**, *4*, 781-791), as well as the fungal metabolites, bulgarein (Fujii et al., *J. Biol. Chem.* **1993**, *268*, 13160-13165) and saintopin (Yamashita et al., *Biochemistry* **1991**, *30*, 5838-5845) and indolocarbazoles (Yamashita et al., *Biochemistry* **1992**, *31*, 12069-12075) have been identified as topoisomerase I poisons. Other topoisomerase poisons have been identified including certain benzo[i]phenanthridine and cinnoline compounds (see LaVoie et al., U.S. Patent No. 6,140,328 and WO 01/32631).

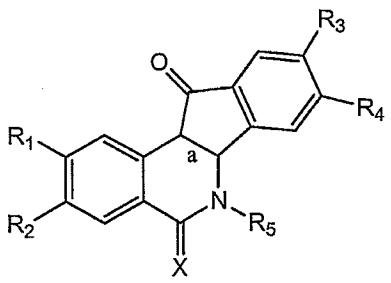
International Patent Application Publication Number 00/21537 discusses certain specific indenoisoquinolines that are reported to have antineoplastic activity.

Despite these reports there is currently a need for additional agents that are useful for treating cancer.

Summary of the Invention

Applicant has discovered compounds that show inhibitory activity against topoisomerase I and/or topoisomerase II, and compounds that are effective cytotoxic agents against cancer cells, including drug-resistant cancer cells. In particular, Applicant has discovered that substitution of a nitro, amino, or a substituted amino group for either one or more of the methoxyl groups or the methylenedioxy groups of tetracyclic topoisomerase I-targeting agents unexpectedly provides compounds with high and potent cytotoxic activity.

Accordingly, the invention provides a compound of the invention which is a compound of formula I:



(I)

wherein:

one of R_1 and R_2 is nitro or NR_aR_b; the other of R_1 and R_2 is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d; and R_3 and R_4 are each independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d, or R_3 and R_4 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy; or

R_1 and R_2 are each independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d, or R_1 and R_2 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy; one of R_3 and R_4 is nitro or NR_aR_b; and the other of R_3 and R_4 is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d;

R_5 is $(C_1\text{-}C_6)$alkyl substituted with one or more solubilizing groups;

X is two hydrogens, =O, =S, or $=NR_e$;

the bond marked "a" is a single bond or a double bond:

R_a and R_b are each independently hydrogen or $(C_1\text{-}C_6)$alkyl, or R_a and R_b together with the nitrogen to which they are attached form a pyrrolidino, piperidino or morpholino ring;

each R_c is hydrogen, $(C_1\text{-}C_6)$alkyl, aryl, or aryl$(C_1\text{-}C_6)$alkyl;

each R_d is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkanoyl, aryl, or aryl$(C_1\text{-}C_6)$alkyl; and

R_e is hydrogen, $(C_1\text{-}C_6)$alkyl, aryl, or aryl$(C_1\text{-}C_6)$alkyl;

or a pharmaceutically acceptable salt thereof.

The invention also provides a pharmaceutical composition comprising a effective amount of a compound of the invention in combination with a pharmaceutically acceptable diluent or carrier.

The invention also provides a method of inhibiting cancer cell growth, comprising administering to a mammal afflicted with cancer, an amount of a compound of the invention, effective to inhibit the growth of said cancer cells.

The invention also provides a method comprising inhibiting cancer cell growth by contacting said cancer cell *in vitro* or *in vivo* with an amount of a compound of the invention, effective to inhibit the growth of said cancer cell.

The invention also provides a compound of the invention for use in medical therapy, preferably for use in treating cancer, for example, solid tumors, as well as the use of a compound of the invention for the manufacture of a medicament useful for the treatment of cancer, for example, solid tumors.

The invention also provides processes and novel intermediates disclosed herein which are useful for preparing compounds of the invention. Some of the compounds of formula I are useful to prepare other compounds of formula I.

Brief Description of the Figures

Figure 1 illustrates the synthesis of representative compounds of formula I (**4a**, **4b**, **5a**, and **5b**).

Figure 2 illustrates the synthesis of representative compounds of formula I (**9a**, **9b**, **10a**, and **10b**).

Figure 3 illustrates the synthesis of representative compounds of formula I (**13a**, **13b**, **14a**, **14b**, **15a**, **15b**, **16a**, and **16b**).

Figure 4 illustrates the synthesis of representative compounds of formula I (**19a**, **19b**, **20a**, **20b**, **21a**, **21b**, **22a**, and **22b**)

Figure 5 illustrates the synthesis of a representative

compounds of formula I (Compound A and Compound B)

Detailed Description

The following definitions are used, unless otherwise described.

"(C_1-C_6)alkyl" denotes both straight and branched carbon chains with 1, 2, 3, 4, 5, or 6, carbon atoms, but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to.

"(C_3-C_6)cycloalkyl" denotes a carbocyclic ring with 3, 4, 5, or 6, carbon atoms.

"Aryl" denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic. Examples of aryl include phenyl, indenyl, and naphthyl.

"Aryl(C_1-C_6)alkyl" refers to a group of the formula aryl-(C_1-C_6)alkyl-, where aryl and (C_1-C_6)alkyl are as defined herein.

1. "Solubilizing group (R_z)" is a substituent that increases the water solubility of the compound of formula I compared to the corresponding compound lacking the R_z substituent (i.e. wherein R_z is hydrogen). Examples of solubilizing groups include (C_1-C_6)alkoxycarbonyl (e.g. -CO_2Me), cyano, halo, hydroxy, mercapto, oxo (=O), carboxy (COOH), nitro, pyrrolidinyl, piperidinyl, imidazolidinyl, imidazolinyl, piperazinyl, morpholinyl, thiomorpholinyl, and -NR_fR_g, wherein R_f and R_g may be the same or different and are chosen from hydrogen, (C_1-C_6)alkyl, and (C_3-C_6)cycloalkyl.

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

A specific value for R_1 is nitro or NR_aR_b.

A specific value for R_1 is nitro.

A specific value for R_1 is NR_aR_b.

A specific value for R_2 is hydrogen, or OR_d, wherein each R_d is hydrogen or $(C_1\text{-}C_6)$alkyl.

A specific value for R_2 is hydrogen.

A specific value for R_2 is nitro or NR_aR_b.

A specific value for R_2 is nitro.

A specific value for R_2 is NR_aR_b.

A specific value for R_1 is hydrogen, or OR_d, wherein each R_d is hydrogen or $(C_1\text{-}C_6)$alkyl.

A specific value for R_1 is hydrogen.

A specific value for R_3 is nitro or NR_aR_b.

A specific value for R_3 is nitro.

A specific value for R_3 is NR_aR_b.

A specific value for R_4 is hydrogen, or OR_d, wherein each R_d is hydrogen or $(C_1\text{-}C_6)$alkyl.

A specific value for R_4 is hydrogen.

A specific value for R_4 is nitro or NR_aR_b.

A specific value for R_4 is nitro.

A specific value for R_4 is NR_aR_b.

A specific value for R_3 is hydrogen, or OR_d, wherein each R_d is hydrogen or $(C_1\text{-}C_6)$alkyl.

A specific value for R_3 is hydrogen.

A specific compound is a compound wherein R_3 and R_4 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy.

A specific compound is a compound wherein R_3 and R_4 taken together are methylenedioxy.

A specific compound is a compound wherein R_1 and R_2 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy.

A specific compound is a compound wherein R_1 and R_2 taken together are methylenedioxy.

A specific compound is a compound wherein R_1 and R_2 are each independently OR_d, wherein each R_d is hydrogen or (C_1-C_6)alkyl.

A specific compound is a compound wherein R_1 and R_2 are each methoxy.

A specific compound is a compound wherein R_3 and R_4 are each independently OR_d, wherein each R_d is hydrogen or (C_1-C_6)alkyl.

A specific compound is a compound wherein R_3 and R_4 are each methoxy.

A specific value for R_5 is (C_1-C_6)alkyl substituted with one or more hydroxy groups.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one hydroxy group.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one or more mercapto groups.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one mercapto group.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one or more carboxy groups.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one carboxy group.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one or more NR_fR_g groups.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one NR_fR_g group.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one or more NH_2 groups.

Another specific value for R_5 is a (C_1-C_6)alkyl substituted with one NH_2 group.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one or more $N(CH_3)_2$ groups.

Another specific value for R_5 is a (C_1-C_6)alkyl substituted with one $N(CH_3)_2$ group.

Another specific value for R_5 is (C_1-C_6)alkyl substituted with one or more $N(CH_2CH_3)_2$ groups.

Another specific value for R_5 is a (C_1-C_6)alkyl substituted with one $N(CH_2CH_3)_2$ group.

Another specific value for R_5 is a (C_1-C_6)alkyl substituted with one or more (C_1-C_6)alkoxycarbonyl (e.g. $-CO_2Me$), cyano, halo, hydroxy, mercapto, oxo (=O), carboxy (COOH), nitro, pyrrolidinyl, piperidinyl, imidazolidinyl, imidazolinyl, piperazinyl, morpholinyl, thiomorpholinyl, or $-NR_fR_g$ groups, wherein R_f and R_g may be the same or different and are chosen from hydrogen, (C_1-C_6)alkyl, and (C_3-C_6)cycloalkyl.

Another specific value for R_5 is a (C_2-C_4)alkyl substituted with one or two groups selected from hydroxy, mercapto, carboxy, amino, methylamino, ethylamino, dimethylamino, and diethylamino.

Another specific value for R_5 is 2-hydroxyethyl.

Another specific value for R_5 is 3-hydroxypropyl.

Another specific value for R_5 is 2-hydroxypropyl.

Another specific value for R_5 is $-CH_2CH_2-NR_fR_g$ or $-CH_2CH_2CH_2-NR_fR_g$ wherein R_f and R_f are each independently hydrogen or (C_1-C_6)alkyl.

Another specific value for R_5 is $-CH_2CH_2-NR_fR_g$ or $-CH_2CH_2CH_2-NR_fR_g$ wherein R_f and R_g are each independently methyl or ethyl.

A specific compound is a compound wherein R_1 is hydrogen; R_2 is nitro; and R_3 and R_4 taken together are methylenedioxy.

A specific compound is a compound wherein R_1 is nitro; R_2 is hydrogen; and R_3 and R_4 taken together are methylenedioxy.

A specific compound is a compound wherein R_1 and R_2 are each methoxy; R_3 is nitro; and R_4 is hydrogen.

A specific compound is a compound wherein R_1 and R_2 are each methoxy; R_3 is hydrogen; and R_4 is nitro.

A specific compound is a compound wherein R_1 is hydrogen; R_2 is nitro; and R_3 and R_4 are each methoxy.

A specific compound is a compound wherein R_1 is nitro; R_2 is hydrogen; and R_3 and R_4 are each methoxy.

A specific compound is a compound wherein R_1 and R_2 taken together are methylenedioxy; R_3 is nitro; and R_4 is hydrogen.

A specific compound is a compound wherein R_1 and R_2 taken together are methylenedioxy; R_3 is hydrogen; and R_4 is nitro.

A specific compound is a compound wherein the bond marked "**a**" is a single bond (i.e. a compound of formula (III).

A specific compound is a compound wherein the bond marked "**a**" is a single bond and the ring juncture at this bond is *cis*.

A specific compound is a compound wherein the bond marked "**a**" is a single bond and the ring juncture at this bond is *trans*.

A specific compound is a compound wherein the bond marked "**a**" is a double bond (i.e. a compound of formula (II).

A specific compound is any one of compounds **4a**, **4b**, **8a**, and **8b** wherein R_5 is 2-(*N*,*N*-dimethylamino)ethyl, 2-(*N*,*N*-dimethylamino)propyl, 2-(*N*,*N* -diethylamino)ethyl, or 2-(*N*,*N* -diethylamino)propyl; or a pharmaceutically acceptable salt thereof.

A specific compound is any one of compounds **4a**, **4b**, **9a**, and **9b** wherein R_5 is 2-(*N*,*N*-dimethylamino)ethyl, 2-(*N*,*N*-dimethylamino)propyl, 2-(*N*,*N* -diethylamino)ethyl, or 2-(*N*,*N* -diethylamino)propyl; or a pharmaceutically acceptable salt thereof.

A specific compound is any one of compounds **10a** and **10b** wherein R_5 is 2-(*N*,*N*-dimethylamino)ethyl, 2-(*N*,*N*-dimethylamino)propyl, 2-(*N*,*N* -diethylamino)ethyl, or 2-(*N*,*N* -diethylamino)propyl; or a pharmaceutically acceptable salt thereof.

A specific compound is any one of compounds **14a**, **14b**, **16a**, and **16b** wherein n is 1, 2, or 3; and Y is dimethylamino or diethylamino; or a pharmaceutically acceptable salt thereof.

A specific compound is any one of compounds **20a**, **20b**, **22a**, and **22b** wherein n is 1, 2, or 3; and Y is dimethylamino or diethylamino; or a pharmaceutically acceptable salt thereof.

A compound of formula I can be prepared using procedures similar to those described in International Patent Application Publication Number 00/21537 or as illustrated in Figure 1. Methods for the preparation of **1** have been described (see *J. Chem. Soc.*, **1955**, 2675-2685). Reaction of the appropriate benzaldehyde with a primary amine (R_5-NH_2) provides the Shiff base intermediates (**2a** and **2b**). Reaction of **2a** or **2b** with homophthalic anhydride **1** provides the 4-carboxy-N-substituted-3,4-dihydro-3-phenyl-2*H*-isoquinolin-1-ones (**3a** and **3b**). Treatment with thionyl chloride provides the compounds of formula (I) (**4a** and **4b**). Alternate treatment with Eaton's reagent (10% P_2O_5 in methanesulfonic acid) instead of thionyl chloride provides the compounds of formula (I) (**5a** and **5b**).

Compounds of formula I can also be prepared as illustrated in Figure 2. Reaction of 3,4-methylenedioxybenzaldehyde with a primary amine (R_5-NH_2) provides the Shiff base intermediate **7**. Reaction **6a** or **6b** with compound **7** provides cis-4-carboxy-3,4-dihydro-N-substituted-3-(3',4'-methylenedioxypheny)-1(2*H*)isoquinolones **8a** and **8b**. Treatment with thionyl chloride provides compounds **9a** and **9b**. Alternate treatment with Eaton's reagent (10% P_2O_5 in methanesulfonic acid) instead of thionyl chloride provides the compounds of formula (I) (**10a** and **10b**).

Compounds of formula I can also be prepared as illustrated in Figure 3. Reaction of compound **1** with compound **11a** or **11b** (wherein X is, for example, hydroxy, protected hydroxy, halo, or cyano) provides compounds **12a** or **12b**, which can be cyclized to provide compounds **13a** or **13b**, which are compounds of formula (I). Subsequent conversion, for example of a compound wherein X is

halo, provides additional compounds of formula (I) (compounds **14a** or **14b**) wherein Y is NR_aR_b or $CH_2NR_aR_b$. Alternate treatment of compounds **12a** or **12b** with Eaton's reagent (10% P_2O_5 in methanesulfonic acid) instead of thionyl chloride provides the compounds of formula (I) (**15a** and **15b**). Subsequent conversion, for example of a compound wherein X is halo, provides additional compounds of formula (I) (compounds **16a** or **16b**) wherein Y is NR_aR_b or $CH_2NR_aR_b$.

Compounds of formula I can also be prepared as illustrated in Figure 4. Reaction of compound **5a** or **5b** with compound **17** (wherein X is, for example, hydroxy, protected hydroxy, halo, or cyano) provides compounds **18a** or **18b**, which can be cyclized to provide compounds **19a** or **19b**, which are compounds of formula (I). Subsequent conversion, for example of a compound wherein X is halo, provides additional compounds of formula (I) (compounds **20a** or **20b**) wherein Y is NR_aR_b or $CH_2R_aR_b$. Alternate treatment of compounds **18a** or **18b** with Eaton's reagent (10% P_2O_5 in methanesulfonic acid) instead of thionyl chloride provides the compounds of formula (I) (**21a** and **21b**). Subsequent conversion, for example of a compound wherein X is halo, provides additional compounds of formula (I) (compounds **22a** or **22b**) wherein Y is NR_aR_b or $CH_2NR_aR_b$.

Compounds wherein R_1-R_4 are amino can be prepared from the corresponding compounds wherein R_1-R_4 are nitro by reduction of the nitro group using procedures that are known, such as, for example, with Rainy nickel and hydrazine. Additionally, standard methods can be used to substitute the resulting aryl amines to provide additional compounds of the invention.

The starting materials employed in the synthetic methods described herein are commercially available, have been reported in the scientific literature, or can be prepared from readily available starting materials using procedures known in the field. It may be desirable to optionally use a protecting group during all or portions of the above described synthetic procedures. Such protecting groups and methods for their introduction and removal are well

known in the art. See Greene, T.W.; Wutz, P.G.M. "Protecting Groups In Organic Synthesis" second edition, 1991, New York, John Wiley & Sons, Inc.

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine topoisomerase inhibition activity or cytotoxic activity using the standard tests described herein, or using other similar tests which are well known in the art.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal, for example, sodium, potassium or lithium, or alkaline earth metal, for example calcium, salts of carboxylic acids can also be made.

The compounds of formula I can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in

a variety of forms adapted to the chosen route of administration, that is, orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

Thus, the present compounds may be systemically administered, for example, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and

substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization.

In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds of formula I to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the compound(s) of formula I in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg per day, e.g., from about 1 to about 60 mg/kg of body weight per day or about 2 to 50 mg/kg per day.

The compound may conveniently be administered in unit dosage form; for example, containing 5 to 1,000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

The ability of a compound of the invention to effect topoisomerase I or II mediated DNA cleavage can be determined using pharmacological models that are well known to the art, for example, using a model like Test A described below.

Test A. **Topoisomerase-mediated DNA cleavage assays.**

Human topoisomerase I was expressed in *E. Coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously (Gatto, B., Sanders, M. M., Yu, C., Wu, H.-Y., Makhey, D., LaVoie, E. J., and

Liu, L. F. (1996) *Cancer Res. 56*, 2795-2800). Recombinant human topoisomerase IIα was isolated and purified as previously described (Wasserman, R.A. Austin, C.A., Fisher, L.M.; Wang, J. C., *Cancer Res.*, **1993**, *53*, 3591; Halligan, B. D.; Edwards, K. A.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 2475). Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described. The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described (Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning, a Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY 1982; pp 149-185.). The cleavage assays were performed as previously reported (Gatto, B., Sanders, M. M., Yu, C., Wu, H.-Y., Makhey, D., LaVoie, E. J., and Liu, L. F. (1996) *Cancer Res. 56*, 2795-2800; Tewey, K. M., Rowe, T. C., Yang, L., Hallogan, B. C., and Liu, L. F. (1984) *Science 226*, 466-468; Li T-K., Chen AY, Yu C, Mao Y, Wang H, Liu LF. (1999) Genes Dev 13(12):1553-60; Wang, H.; Mao, Y.; Chen, A.Y.; Zhou, N.; and LaVoie, E.J.; Liu, L.F. *Biochemistry*, **2001**, *40*, 3316). The drug and the DNA in presence of topoisomerase I was incubated for 30 minutes at 37 °C. After development of the gels, typically 24-hour exposure was used to obtain autoradiograms outlining the extent of DNA fragmentation. Topoisomerase I-mediated DNA cleavage values are reported as REC, Relative Effective Concentration, i.e. concentrations relative to topotecan, whose value is arbitrarily assumed as 1.0, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I. Topoisomerase II-mediated DNA cleavage values are reported as REC, Relative Effective Concentration, potency was based upon the relative amount of drug needed to induce approximately 10% DNA fragmentation, i.e. concentrations relative to VM-26, whose value is arbitrarily assumed as 1.0, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase II.

The cytotoxic effects of a compound of the invention can be determined using pharmacological models that are well known to the art, for example, using a model like Test B described below.

Test B. **Inhibition of Cell Growth: MTT-microtiter plate tetrazolinium cytotoxicity assay (RPMI 8402, CPT-K5, U937, U937/CR Cells)**

The cytotoxicity is determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA), see Chen A.Y. et al. *Cancer Res.* **1993**, *53*, 1332; Mosmann, T. J., J. *Immunol. Methods* **1983**, *65*, 55; and Carmichael, J. et al. *Cancer Res.* **1987**, *47*, 936. The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Anchi Cancer Research Institute, Nagoya, Japan), see Andoh, T.; Okada, K, *Adv. in Pharmacology* **1994**, *29B*, 93. Human U-937 myeloid leukemia cells and U-937/CR cells were described by Rubin et al., *J. Biol. Chem.*, **1994**, *269*, 2433-2439. The cytotoxicity assay is performed by using 96-well microtiter plates using 2000 cells/well, in 200 mL of growth medium. Cells are grown in suspension at 37 °C in 5% CO_2 and maintained by regular passage in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100U/mL), and streptomycin (0.1 mg/mL). For determination of IC_{50}, cells are exposed continuously for 3-4 days to varying concentrations of drug, and MTT assays were performed at the end of the fourth day. Each assay is performed with a control that did not contain any drug. All assays are performed at least twice in 6 replicate wells. All assays are performed under the direction of Dr. L. F. Liu, Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey. Data for representative compound **B** is provided in Table 1.

Table 1

Cell Lines

RPMI8402	CPT-K5	KB3-1	KBV-1	KBH5.0	HL60	HL60/MX2
0.003*	2.4	0.004	0.004	0.004	0.003	0.003

*Cytotoxicity (μM IC_{50} values)

The compounds of the invention can function as cytotoxic agents against tumor cell lines, including multi-drug resistant tumor cell lines. Thus, the compounds are useful to treat cancer and can be used to treat tumors that are resistant to other specific chemotherapeutic agents.

Topoisomerase inhibitors are also known to possess antibacterial, antifungal, antiprotozoal, antihelmetic, antipsoriatic, and antiviral activity. Accordingly, the topoisomerase inhibitors of the invention may also be useful as antibacterial, antifungal, antiprotozoal, antihelmetic, antipsoriatic, or antiviral agents. In particular, compounds of the invention that demonstrate little or no activity as mammalian topoisomerase I poisons, because of the possibility of similar molecular mechanism of action, could be highly active and selective antibacterial, antifungal, antiprotozoal, antihelmetic, antipsoriatic, or antiviral agents. Thus, certain compounds of the invention may be particularly useful as systemic antibacterial, antifungal, antiprotozoal, antihelmetic, antipsoriatic, or antiviral agents in mammals. The invention also provides the use of a compound of the invention for the manufacture of a medicament useful for producing an antibacterial, antifungal, antiprotozoal, antihelmetic, antipsoriatic, or antiviral effect in a mammal.

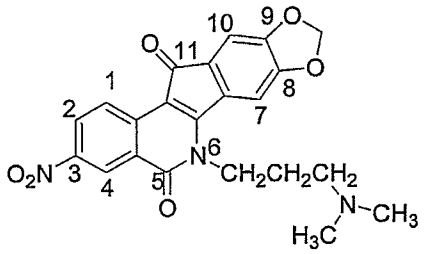
As used herein, the term "solid mammalian tumors" include cancers of the head and neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, rectum, anus, kidney, ureter, bladder, prostate, urethra, penis, testis, gynecological organs, ovarian, breast, endocrine system, skin central nervous system; sarcomas of the soft tissue and bone; and melanoma of cutaneous and intraocular origin. The term "hematological malignancies" includes childhood leukemia and lymphomas, Hodgkin's disease, lymphomas of lymphocytic and cutaneous origin, acute and

chronic leukemia, plasma cell neoplasm and cancers associated with AIDS. The preferred mammalian species for treatment are humans and domesticated animals.

The invention will now be illustrated by the following non-limiting Example.

Examples

Example 1. 6-[3-(N,N-dimethylamino)propyl]-3-Nitroindeno[1,2-*c*]-indenoisoquinolin-5,11-dione (Compound B, Fugure 5).



The title compound was prepared as illustrated in Figure 5. To a solution of **Compound A** (300 mg, 0.66 mmol) in chloroform (50 mL) and methanol (50 mL) was added a 2.0 M solution of dimethylamine in methanol (6 mL), and the resulting mixture was heated in a steel bomb to 140 °C, and maintained at this temperature with stirring for 48 h. The reaction mixture was cooled to room temperature and the solvent was removed under vacuum. To the residue was added water, and the resulting suspension was basified (10% NaOH), extracted with $CHCl_3$, and chromatographed on silica eluting with 95:5 chloroform-methanol, to provide 60 mg of the title compound as a red solid, in 21% yield; mp 191-192 °C; ^{1}H NMR ($CDCl_3$) 2.04 (m, 2H), 2.38 (s, 6H), 2.58 (m, 2H), 4.57 (t, 2H, *J*=7.9), 6.16 (s, 2H), 7.18 (s, 1H), 7.72 (s, 1H), 8.45 (dd, 1H, *J*=9.1, *J*=2.3), 8.76 (d, 1H, *J*=9.1), 9.17 (d, 1H, *J*=2.3); IR (KBr) 1697, 1674, 1499, 1337; HRMS calcd for $C_{22}H_{19}N_3O_6H$: 422.1352; found: 422.1357.

The intermediate Compound A was prepared as follows.

a. **Compound A.** 4-Nitrohomophthalic anhydride (4.14 g, 20.0 mmol, see Whitmore, W.F., et al., *J. Am. Chem. Soc.*, **1944**, *66*, 1237-1240) was added to a solution of 3,4-methylenedioxybenzylidene-(3-bromo-1-propylamine) (5.4 g, 20.0 mmol, see Cushman, M., et al., *J. Med. Chem.*, **2000**, *43*, 3688-3698) in chloroform (200 mL), and the resulting mixture was stirred at room temperature overnight. The material that precipitated during the course of the reaction was isolated by filtration and was washed with chloroform. Drying yielded 6.3 g of material containing of a mixture of isomers. The mixture not characterized or purified further at this stage. 3.0 g (6.3 mmol) of this material was added to 12 mL of thionyl chloride, and the resulting mixture was stirred at room temperature overnight. Benzene was added to the red solution and it was concentrated under reduced pressure. Chloroform was added to the residue and the mixture was filtered through a short column of silica, providing 350 mg of a dark brown solid, in 8% yield; mp 281-282 °C; ^{1}H NMR ($CDCl_3$) 2.19 (m, 2H), 3.69 (t, 2H, *J*=6.0), 4.66 (t, 2H, *J*=8.1), 6.18 (s, 2H), 7.21 (s, 1H), 7.49 (s, 1H), 8.49 (dd, 1H, *J*=9.2, *J*=2.6), 8.79 (d, 1H, *J*=9.2), 9.18 (d, 1H, *J*=2.6); IR (KBr) 1698, 1658, 1504, 1333; HRMS calcd for $C_{20}H_{13}N_2O_6BrH$: 457.0037; found: 457.0035.

Example 2 The following illustrate representative pharmaceutical dosage forms, containing a compound of formula I ('Compound X'), for therapeutic or prophylactic use in humans.

(i) Tablet 1	mg/tablet
'Compound X'	100.0
Lactose	77.5
Povidone	15.0
Croscarmellose sodium	12.0
Microcrystalline cellulose	92.5
Magnesium stearate	3.0
	300.0

(ii) Tablet 2	mg/tablet
'Compound X'	20.0
Microcrystalline cellulose	410.0
Starch	50.0
Sodium starch glycolate	15.0
Magnesium stearate	5.0
	500.0

(iii) Capsule	mg/capsule
'Compound X'	10.0
Colloidal silicon dioxide	1.5
Lactose	465.5
Pregelatinized starch	120.0
Magnesium stearate	3.0
	600.0

(iv) Injection 1 (1 mg/ml)	mg/ml
'Compound X' (free acid form)	1.0
Dibasic sodium phosphate	12.0
Monobasic sodium phosphate	0.7
Sodium chloride	4.5
1.0 N Sodium hydroxide solution (pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

(v) Injection 2 (10 mg/ml)	mg/ml
'Compound X' (free acid form)	10.0
Monobasic sodium phosphate	0.3
Dibasic sodium phosphate	1.1
Polyethylene glycol 400	200.0
01 N Sodium hydroxide solution (pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

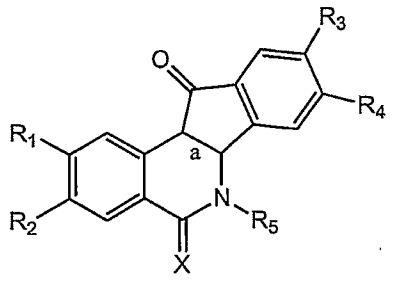
(vi) Aerosol	mg/can
'Compound X'	20.0
Oleic acid	10.0
Trichloromonofluoromethane	5,000.0
Dichlorodifluoromethane	10,000.0
Dichlorotetrafluoroethane	5,000.0

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

What is claimed is:

1. A compound of formula I:



(I)

wherein:

one of R_1 and R_2 is nitro or NR_aR_b; the other of R_1 and R_2 is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d; and R_3 and R_4 are each independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d, or R_3 and R_4 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy; or

R_1 and R_2 are each independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d, or R_1 and R_2 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy; one of R_3 and R_4 is nitro or NR_aR_b; and the other of R_3 and R_4 is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, NR_aR_b, $COOR_c$, or OR_d;

R_5 is $(C_1\text{-}C_6)$alkyl substituted with one or more solubilizing groups;

X is two hydrogens, =O, =S, or $=NR_e$;

the bond marked "a" is a single bond or a double bond:

R_a and R_b are each independently hydrogen or $(C_1\text{-}C_6)$alkyl, or R_a and R_b together with the nitrogen to which they are attached form a pyrrolidino, piperidino or morpholino ring;

each R_c is hydrohgen, $(C_1\text{-}C_6)$alkyl, aryl, or aryl$(C_1\text{-}C_6)$alkyl;

each R_d is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkanoyl, aryl, or aryl$(C_1\text{-}C_6)$alkyl; and

R_e is hydrogen, $(C_1\text{-}C_6)$alkyl, aryl, or aryl$(C_1\text{-}C_6)$alkyl;
or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein R_1 is nitro or NR_aR_b.

3. The compound of claim 1 wherein R_1 is nitro.

4. The compound of any one of claims 1-3 wherein R_1 is NR_aR_b.

5. The compound of any one of claims 1-4 wherein R_2 is hydrogen, or OR_d, wherein each R_d is hydrogen or $(C_1\text{-}C_6)$alkyl.

6. The compound of any one of claims 1-4 wherein R_2 is hydrogen.

7. The compound of claim 1 wherein R_2 is nitro or NR_aR_b.

8. The compound of claim 1 wherein R_2 is nitro.

9. The compound of any one of claims 1-4 wherein R_2 is NR_aR_b.

10. The compound of any one of claims 1-4, 8 and 9 wherein R_1 is hydrogen, or OR_d, wherein each R_d is hydrogen or $(C_1\text{-}C_6)$alkyl.

11. The compound of any one of claims 7-9 wherein R_1 is hydrogen.

12. The compound of claim 1 wherein R_3 is nitro or NR_aR_b.

13. The compound of claim 1 wherein R_3 is nitro.

14. The compound of any one of claims 1-11 wherein R_3 is NR_aR_b.

15. The compound of any one of claims 1- 14 wherein R_4 is hydrogen, or OR_d, wherein each R_d is hydrogen or (C_1-C_6)alkyl.

16. The compound of any one of claims 1- 14 wherein R_4 is hydrogen.

17. The compound of claim 1 wherein R_4 is nitro or NR_aR_b.

18. The compound of claim 1 wherein R_4 is nitro.

19. The compound of any one of claims 1-11 wherein R_4 is NR_aR_b.

20. The compound of any one of claims 1-11, 18, and 19 wherein R_3 is hydrogen, or OR_d, wherein each R_d is hydrogen or (C_1-C_6)alkyl.

21. The compound of any one of claims 1-1, 18, and 19 wherein R_3 is hydrogen.

22. The compound of any one of claims 1-11 wherein R_3 and R_4 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy.

23. The compound of any one of claims 1-11 wherein R_3 and R_4 taken together are methylenedioxy.

24. The compound of any one of claims 1 and 13-23 wherein R_1 and R_2 taken together are methylenedioxy, 1,2-ethylenedioxy, or 1,3-propylenedioxy.

25. The compound of any one of claims 1 and 13-23 wherein R_1 and R_2 are each independently OR_d, wherein each R_d is hydrogen or (C_1-C_6)alkyl.

26. The compound of any one of claims 1 and 13-23 wherein R_1 and R_2 are each methoxy.

27. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one or more hydroxy groups.

28. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one hydroxy group.

29. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one or more mercapto groups.

30. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one mercapto group.

31. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one or more carboxy groups.

32. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one carboxy group.

33. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one or more NR_fR_g groups.

34. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one NR_fR_g group.

35. The compound of any one of claims 1-26 wherein R_5 is $(C_1$-$C_6)$alkyl substituted with one or more NH_2 groups.

36. The compound of any one of claims 1-26 wherein R_5 is a (C_1-C_6)alkyl substituted with one NH_2 group.

37. The compound of any one of claims 1-26 wherein R_5 is (C_1-C_6)alkyl substituted with one or more $N(CH_3)_2$ groups.

38. The compound of any one of claims 1-26 wherein R_5 is a (C_1-C_6)alkyl substituted with one $N(CH_3)_2$ group.

39. The compound of any one of claims 1-26 wherein R_5 is (C_1-C_6)alkyl substituted with one or more $N(CH_2CH_3)_2$ groups.

40. The compound of any one of claims 1-26 wherein R_5 is a (C_1-C_6)alkyl substituted with one $N(CH_2CH_3)_2$ group.

41. The compound of any one of claims 1-26 wherein R_5 is a (C_1-C_6)alkyl substituted with one or more (C_1-C_6)alkoxycarbonyl, cyano, halo, hydroxy, mercapto, oxo, carboxy, nitro, pyrrolidinyl, piperidinyl, imidazolidinyl, imidazolinyl, piperazinyl, morpholinyl, thiomorpholinyl, or $-NR_fR_g$ groups, wherein R_f and R_g may be the same or different and are chosen from hydrogen, (C_1-C_6)alkyl, and (C_3-C_6)cycloalkyl.

42. The compound of any one of claims 1-26 wherein R_5 is a (C_2-C_4)alkyl substituted with one or two groups selected from hydroxy, mercapto, carboxy, amino, methylamino, ethylamino, dimethylamino, and diethylamino.

43. The compound of any one of claims 1-26 wherein R_5 is 2-hydroxyethyl.

44. The compound of any one of claims 1-26 wherein R_5 is 3-hydroxypropyl.

45. The compound of any one of claims 1-26 wherein R_5 is 2-hydroxypropyl.

46. The compound of any one of claims 1-26 wherein R_5 is $-CH_2CH_2-NR_fR_g$ or $-CH_2CH_2CH_2-NR_fR_g$ wherein R_f and R_f are each independently hydrogen or (C_1-C_6)alkyl.

47. The compound of any one of claims 1-26 wherein R_5 is $-CH_2CH_2-NR_fR_g$ or $-CH_2CH_2CH_2-NR_fR_g$ wherein R_f and R_f are each independently methyl or ethyl.

48. The compound of claim 1 wherein R_1 is hydrogen; R_2 is nitro; and R_3 and R_4 taken together are methylenedioxy.

49. The compound of claim 1 wherein R_1 is nitro; R_2 is hydrogen; and R_3 and R_4 taken together are methylenedioxy.

50. The compound of claim 1 wherein R_1 and R_2 are each methoxy; R_3 is nitro; and R_4 is hydrogen.

51. The compound of claim 1 wherein R_1 and R_2 are each methoxy; R_3 is hydrogen; and R_4 is nitro.

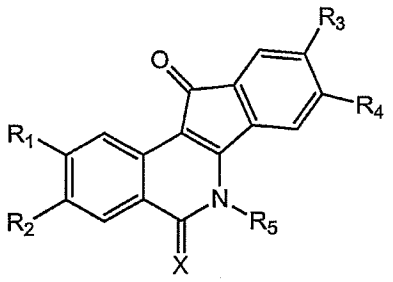
52. The compound of claim 1 wherein R_1 is hydrogen; R_2 is nitro; and R_3 and R_4 are each methoxy.

53. The compound of claim 1 wherein R_1 is nitro; R_2 is hydrogen; and R_3 and R_4 are each methoxy.

54. The compound of claim 1 wherein R_1 and R_2 taken together are methylenedioxy; R_3 is nitro; and R_4 is hydrogen.

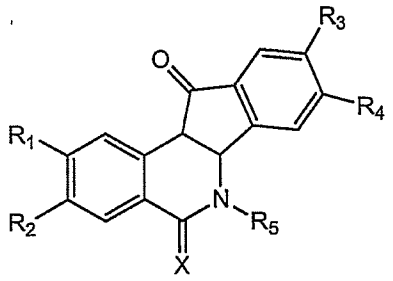
55. The compound of claim 1 wherein R_1 and R_2 taken together are methylenedioxy; R_3 is hydrogen; and R_4 is nitro.

56. The compound of any one of claims 1-55 wherein the compound of formula (I) is a compound of formula (II):



(II).

57. The compound of any one of claims 1-55 wherein the compound of formula (I) is a compound of formula (III):



(III).

58. Any one of compounds **4a**, **4b**, **8a**, and **8b** wherein R_5 is 2-(*N*,*N*-dimethylamino)ethyl, 2-(*N*,*N*-dimethylamino)propyl, 2-(*N*,*N*-diethylamino)ethyl, or 2-(*N*,*N*-diethylamino)propyl or a pharmaceutically acceptable salt thereof.

59. A pharmaceutical composition comprising a compound as described in any one of claims 1-58 in combination with a pharmaceutically acceptable diluent or carrier.

60. A method of inhibiting cancer cell growth, comprising administering to a mammal afflicted with cancer, an amount of a compound as described in any one of claims 1-58, effective to inhibit the growth of said cancer cells.

61. A method comprising inhibiting cancer cell growth by contacting said cancer cell in vitro or in vivo with an amount of a compound as described in any one of claims 1-58, effective to inhibit the growth of said cancer cell.

62. A compound as described in any one of claims 1-58 for use in medical therapy.

63. The compound of claim 62 wherein the therapy is treating cancer.

64. The use of a compound as described in any one of claims 1-58 for the manufacture of a medicament useful for the treatment of cancer.

65. A method of producing an antibacterial effect in a mammal in need of such treatment comprising administering to the mammal, an amount of a compound as described in any one of claims 1-58, effective to provide an antibacterial effect.

66. A method of producing an antifungal effect in a mammal in need of such treatment comprising administering to the mammal, an amount of a compound as described in any one of claims 1-58, effective to provide an antifungal effect.

67. The use of a compound as described in any one of claims 1-58 for the manufacture of a medicament useful for producing an antibacterial, antifungal, antiprotozoal, antihelmetic, antipsoriatic, or antiviral effect in a mammal.

68. The use of a compound as described in any one of claims 1-58 for the manufacture of a medicament useful for producing an antifungal effect in a mammal.

Figure 1

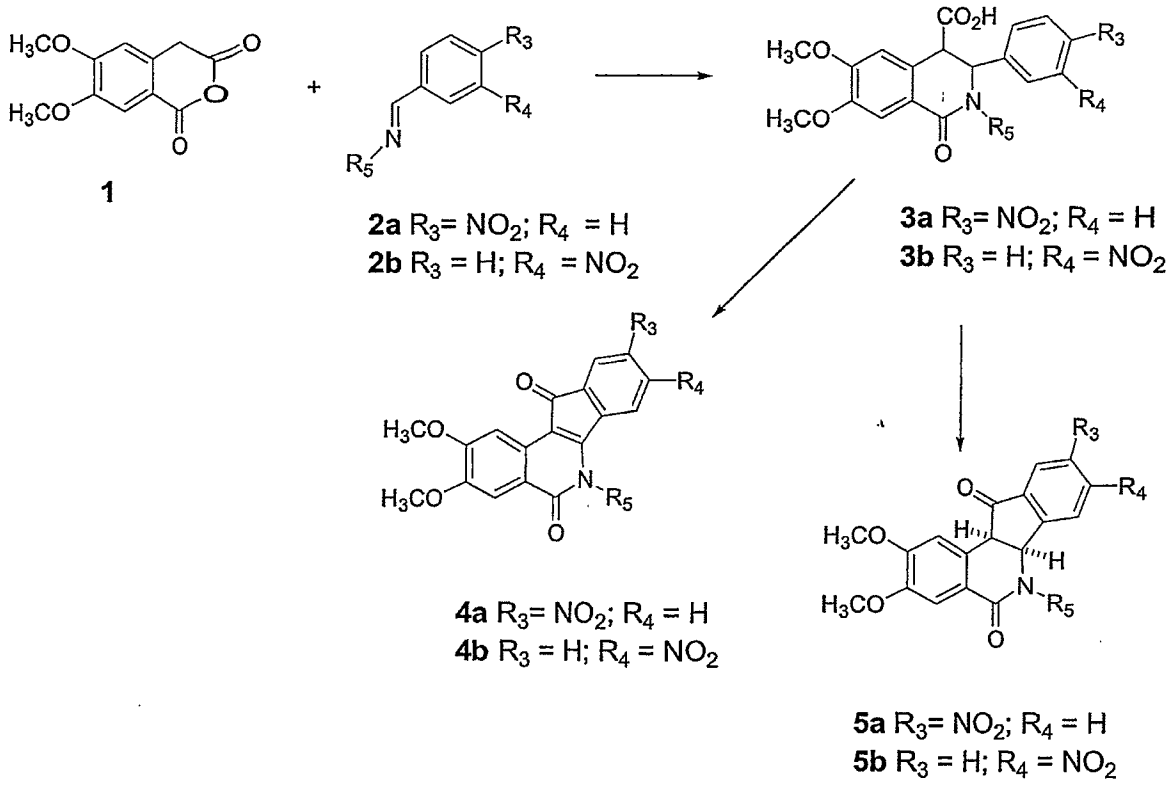

1
2a R_3= NO_2; R_4 = H
2b R_3 = H; R_4 = NO_2
3a R_3= NO_2; R_4 = H
3b R_3 = H; R_4 = NO_2
4a R_3= NO_2; R_4 = H
4b R_3 = H; R_4 = NO_2
5a R_3= NO_2; R_4 = H
5b R_3 = H; R_4 = NO_2

Figure 2

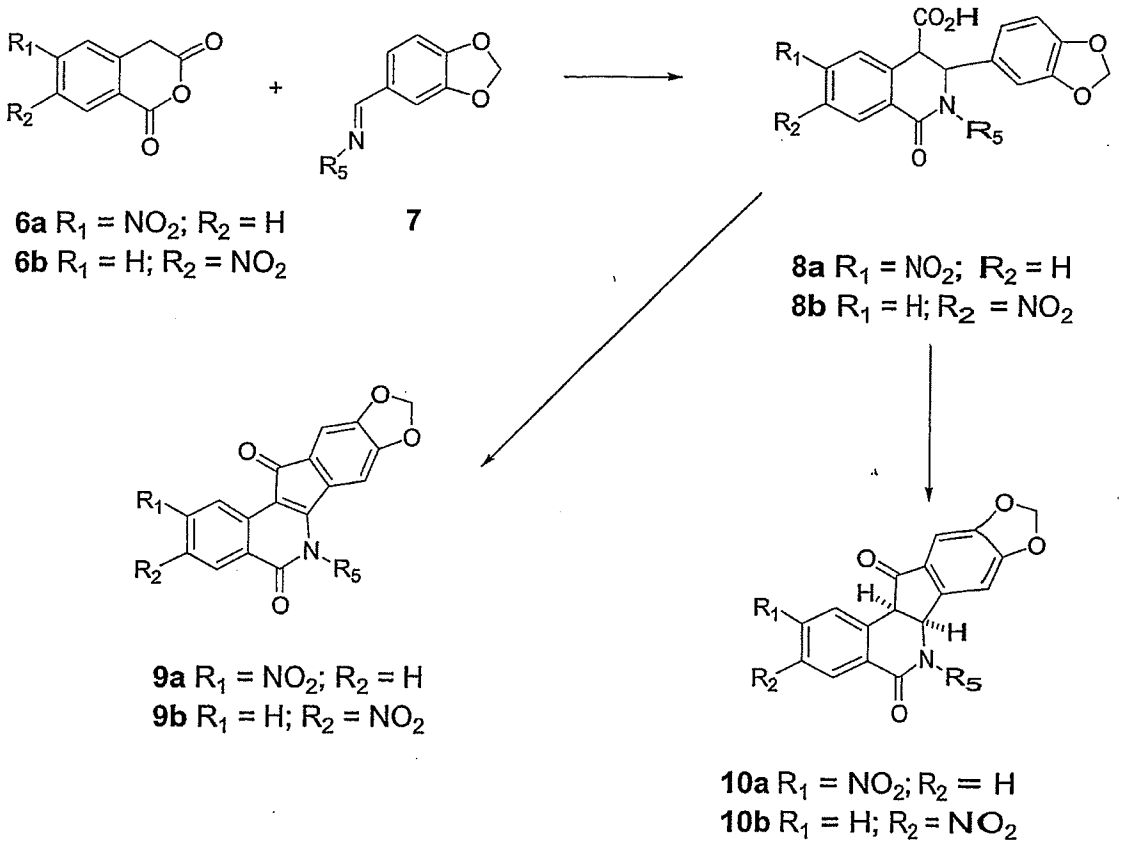
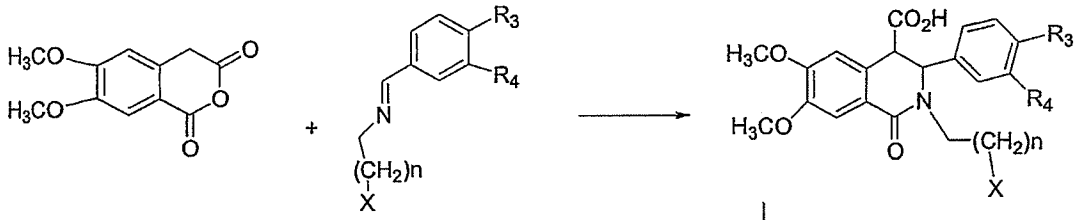

R1
R2
O
O
O
+
N
R5
7
CO2H
N
R5
6a R1 = NO2; R2 = H
6b R1 = H; R2 = NO2
8a R1 = NO2; R2 = H
8b R1 = H; R2 = NO2
9a R1 = NO2; R2 = H
9b R1 = H; R2 = NO2
10a R1 = NO2; R2 = H
10b R1 = H; R2 = NO2

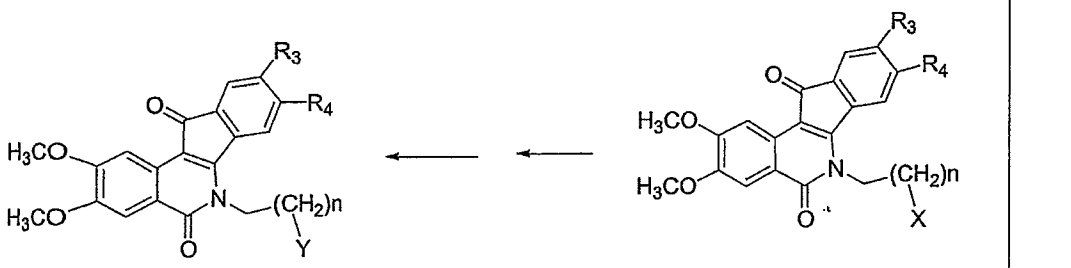
Figure 3



1

11a $R_3 = NO_2$; $R_4 = H$
11b $R_3 = H$; $R_4 = NO_2$

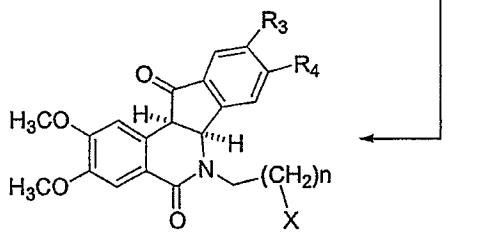
12a $R_3 = NO_2$; $R_4 = H$
12b $R_3 = H$; $R_4 = NO_2$



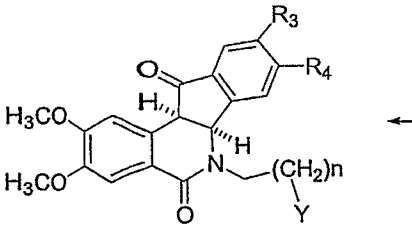
13a $R_3 = NO_2$; $R_4 = H$
13b $R_3 = H$; $R_4 = NO_2$

14a $R_3 = NO_2$; $R_4 = H$
14b $R_3 = H$; $R_4 = NO_2$

Where X = OH, Br, Cl, CN
Where Y = NRaRb or CH_2NRaRb



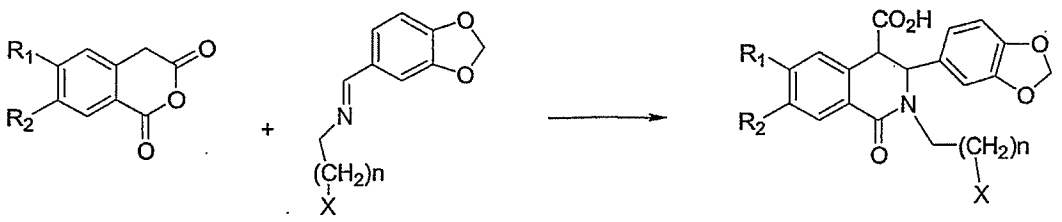
15a $R_3 = NO_2$; $R_4 = H$
15b $R_3 = H$; $R_4 = NO_2$



16a $R_3 = NO_2$; $R_4 = H$
16b $R_3 = H$; $R_4 = NO_2$

Where X = OH, Br, Cl, CN
Where Y = NRaRb or CH_2NRaRb

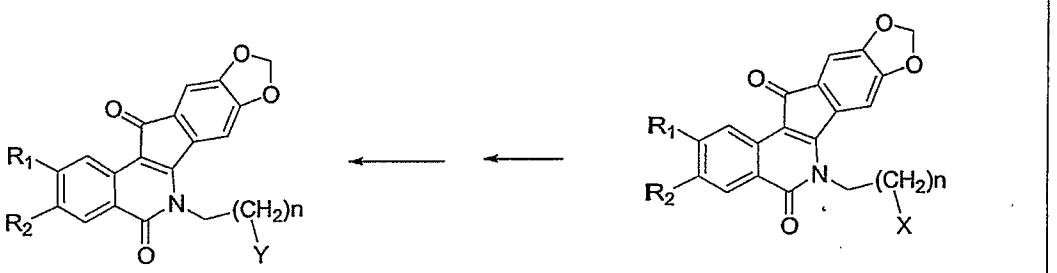
Figure 4



5a $R_1 = NO_2$; $R_2 = H$
5b $R_1 = H$; $R_2 = NO_2$

17

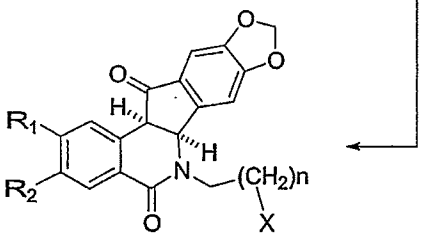
18a $R_1 = NO_2$; $R_2 = H$
18b $R_1 = H$; $R_2 = NO_2$



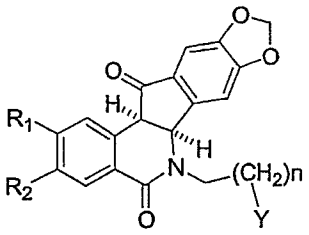
19a $R_1 = NO_2$; $R_2 = H$
19b $R_1 = H$; $R_2 = NO_2$

20a $R_1 = NO_2$; $R_2 = H$
20b $R_1 = H$; $R_2 = NO_2$

Where X = OH, Cl, Br, CN
Where Y = NRaRb or CH_2NRaRb



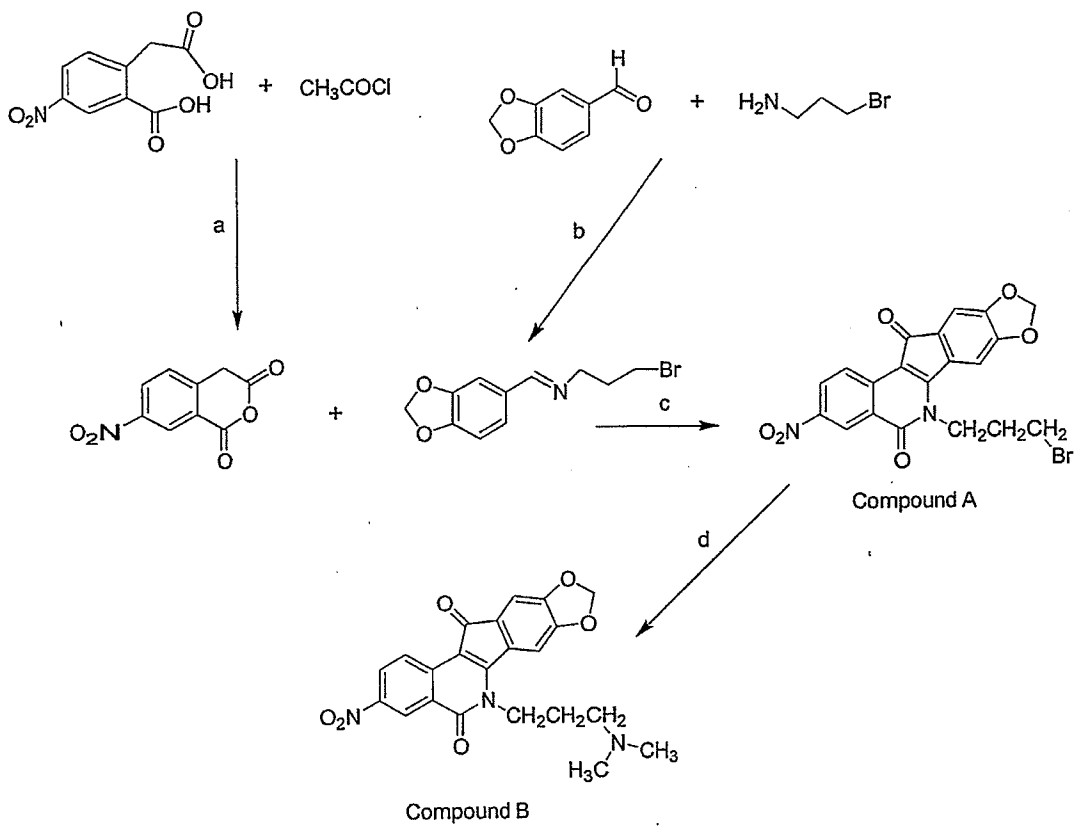
21a $R_1 = NO_2$; $R_2 = H$
21b $R_1 = H$; $R_2 = NO_2$



22a $R_1 = NO_2$; $R_2 = H$
22b $R_1 = H$; $R_2 = NO_2$

Where X = OH, Cl, Br, CN
Where Y = NRaRb or CH_2NRaRb

Figure 5



a) acetone, reflux 4 h; b) $CHCl_3$, TEA, $MgSO_4$ r.t. 16 h; c) $CHCl_3$ at r.t. 12 h, then $SOCl_2$ at r.t. for 12 h; d) MeOH:$CHCl_3$ (1:1), $NH(CH_3)_2$ (2M in MeOH) 140 °C for 48 h

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/25109

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D221/18 C07D491/04 A61K31/4741 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CUSHMAN, MARK ET AL: "Synthesis of New Indeno'1,2-c!isoquinolines: Cytotoxic Non-Camptothecin Topoisomerase I Inhibitors" JOURNAL OF MEDICINAL CHEMISTRY (2000), 43(20), 3688-3698 , XP002263884 the whole document --- -/--	1-68

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 December 2003	19/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schmid, J-C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/25109

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	JAYARAMAN, MUTHUSAMY ET AL: "Synthesis of New Dihydroindeno'1,2-c!isoquinoline and Indenoisoquinoliniu Chloride Topoisomerase I Inhibitors Having High in Vivo Anticancer Activity in the Hollow Fiber Animal Model" JOURNAL OF MEDICINAL CHEMISTRY (2002), 45(1), 242-249 , XP002263891 the whole document	1-68
A	WO 00 21537 A (JAYARAMAN MUTHUSAMY ;NAGAFUJI PAMELA M (US); US HEALTH (US); CUSHM) 20 April 2000 (2000-04-20) the whole document	1-68
A	WO 99 31067 A (LAVOIE EDMOND J ;LIU LEROY FONG (US); UNIV RUTGERS (US); ZHAO BAOP) 24 June 1999 (1999-06-24) the whole document	1-68

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/25109

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 60,65 and 66 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

nation on patent family members

International Application No
PCT/US 03/25109

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 1042294 A1	11-10-2000
		JP 2002508359 T	19-03-2002
		PL 341050 A1	26-03-2001
		WO 9931067 A1	24-06-1999
		US 6486167 B1	26-11-2002